

# p7.5tk

7.5 k promoter

Not I Apa I

GGCCCAAAATTGAAAACTAGATCTATTATTGCACTGGCCGCGCATG GGC CCG GCC GCC AAC AAC GGC GGA  
Met Gly Pro Ala Ala Asn Gly Gly

tk coding sequence



# pE/Ltk

synthetic E/L promoter

Not I Apa I

GGCCCAAAATTGAAATTTTATTTTTTTTGGGAATATATAAGCGGCGCGCATG GGC CCG GCC GCC AAC AAC GGC GGA  
Met Gly Pro Ala Ala Asn Gly Gly

tk coding sequence



A

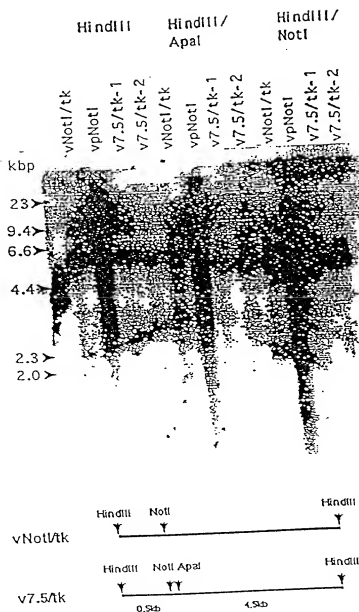


FIGURE 2 A

B

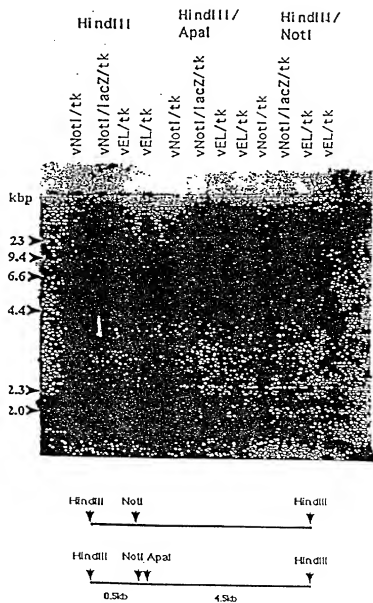


FIGURE 2B

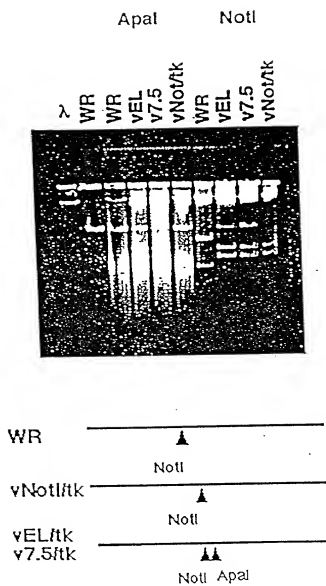
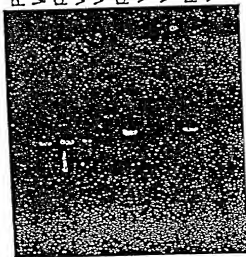


FIGURE 3

Phi X  
WR  
pJNot  
vNotI/tk  
vpNotI  
p7.5/tk  
v7.5/tk  
vNotI/lacZ/tk  
pEL/tk  
vEL/tk



4

# B-Glucoronidase Analysis of Recombinant Viruses

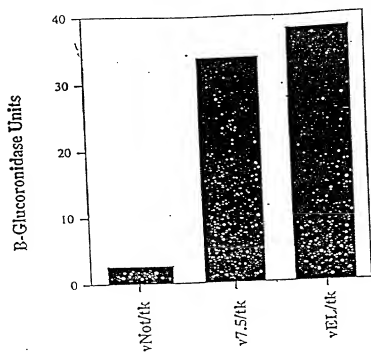


FIGURE 5

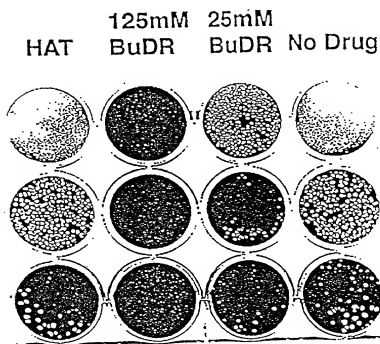


FIGURE 6

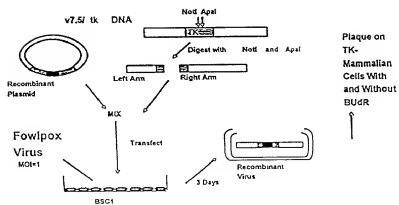


FIGURE 7



1. p7.5tk

7.5K PROMOTER NOTI APAL  
 3'-GGCCAAAAATTGAAAAAAGTAGATCTATTATTGCACGGGGCGGCCCATGGGGCCCGGCC-3'

2. p7.5/ATG0/tk

7.5K PROMOTER NOTI BAMHI SMAI PSTI  
 3'-GGCCAAAAATTGAAAAAAGTAGATCTATTATTGCACGGGGCGGCCGTGGATCCCCGGGGCTGCAGGAA

	TRANSLATION	TRANSCRIPTION
	STOP CODONS	STOP SIGNAL
SALI		
TTTCGATATCAAGCTTATCGATACCGTTCGACCTCGAGGGGGGCGCTAACTAACTAATTTGTTTGT		

APAL  
 GGGCCCGGCC-3'

3. p7.5/ATGI/Hk

7.5K PROMOTER

NOTI

START

CODON BAMHI SMAI PSTI

5'-GGCCAAAATTGAAAAACTAGATCTATTATTGCACGGGGCGCCCA TGGTGGATCCCCCGGGGCTGCAGGAA

SALI

TRANSLATION

TRANSCRIPTION

STOP CODONS

STOP SIGNAL

TTGGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCTAACTAACTTTGTTTTTGT

APAI

GGGGCCCGGCC-3'

4. p7.5/ATG2/tk

7.5K PROMOTER      NOTI      START  
 CODON BAMHI SMAI PSTI

5'. GGCCAAAAATTGAAAACTAGATCTATTATTGCACGGCGGCCcCATGAGTGGATCCCCCGGGCTGCAGGA

SALI      TRANSLATION      TRANSCRIPTION  
 STOP CODONS      STOP SIGNAL

TTTGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGCCTAACTAAATTTGTTTTTGT

APAI

GGGCCCCGGCC-3'

5' p7.5/ATG3/th  
 7.5K PROMOTER  
 NOTI  
 START  
 CODON  
 BAMHI SMAI PSTI  
 5'-GGCCAAAAATTGAAAAAAGTAGATCTATTATTGACCGGGCCGCCATGACGTGGATCCCCCGGGCTGCAGGAA  
 SALI  
 TRANSLATION  
 STOP CODONS  
 TRANSCRIPTION  
 STOP SIGNAL

TTCGATATCAAGCTTATCGATACCGTGGACCTCGAGGGGGGCGCTAACTAACTAAATTTGTTTTGT

APAI  
 GGGCCCCGGCC-3'

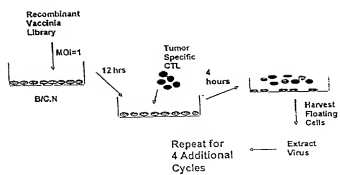


FIGURE 9

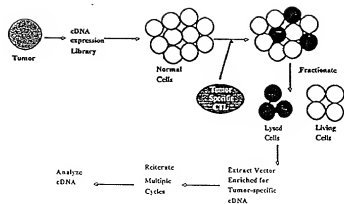


FIGURE 10

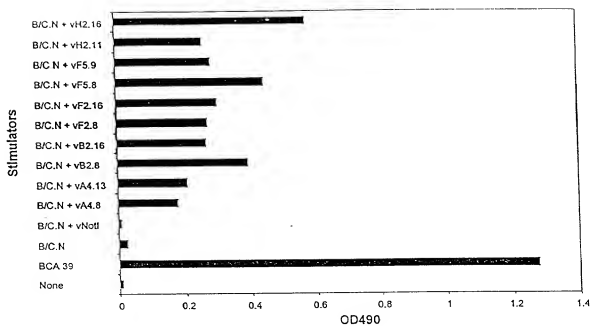


FIGURE 11A

<u>Target</u>	Percent Specific Lysis	
	Effector : Target	
	<u>10:1</u>	<u>2:1</u>
BCA 34	68.4	54.8
BCA 39	36.6	23.4
B/C.N	0.2	0.3
B/C.N + vF5.8	47.5	34.6
B/C.N + vH2.16	67.8	56.2
B/C.N + vaccinia vector	0	0.2

FIGURE 11B



A. L3

Amino Acid Position	45	46	47	48	49	50	51	52	53	54	55	56
Sequence	A	F	L	G	Y	K	A	G	M	T	H	I
Nucleotide	GCC	TTT	CTG	GGT	TAC	AAG	GCT	GGC	ATG	ACC	CAC	ATC

B. H2.16

Amino Acid												
Position	45	46	47	48	49	50	51	52	53	54	55	56
Sequence	A	F	L	G	Y	K	A	G	M	I	H	I
Nucleotide	---	---	---	---	---	---	---	---	---	-T-	---	---

FIGURE 12.

<u>Target</u>	Percent Specific Lysis	
	Effector: Target	
	<u>10:1</u>	<u>2:1</u>
BCA 34	62.4	32.1
BCA 39	49.7	23.6
B/C.N	3.3	0.2
B/C.N + L3 peptide 48-56(I54)	46.0	16.1
B/C.N + L3 peptide 48-56(T54)	2.0	0
B/C.N + L3 peptide 45-54(I54)	0	0

FIGURE 13A

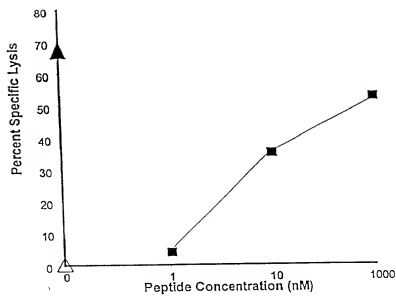
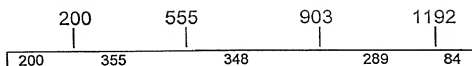


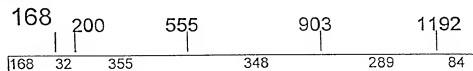
FIGURE 13B

Published L3 (1276 bp)



168-171 = GACC

H2.16 (1276 bp)



168-171 = GATC

FIGURE 14A

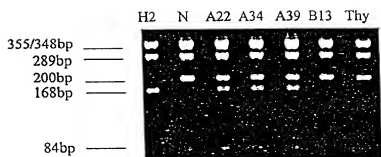


FIGURE 14B

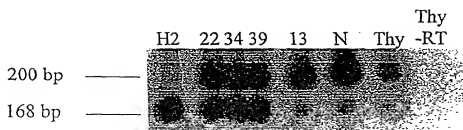


FIGURE 14C

Target	Percent Specific Lysis Immunogen			
	vH2.16		v7.5/tk	
	<u>40:1</u>	<u>10:1</u>	<u>40:1</u>	<u>10:1</u>
BCA 34	33.6	12.9	5.7	4.0
BCA 39	22.1	9.0	5.3	3.1
B/C.N + L3 48-56 (I54)	48.2	20.2	3.9	1.5
B/C.N + L3 48-56 (T54)	6.4	1.4	1.8	2.9
B/C.N	7.1	5.7	6.1	2.8
YAC	1.2	2.5	0	1.8

FIGURE 15 A

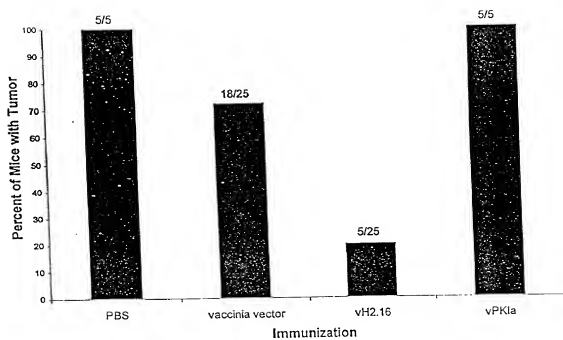


FIGURE 15B



A bar graph titled 'Percent of Mice with Tumor' on the y-axis. The y-axis ranges from 0 to 100 in increments of 10. The x-axis is labeled 'Immunization' and has two categories: 'Vaccinia Vector' and 'vH2.16'. The bar for 'Vaccinia Vector' reaches the 80 mark on the y-axis and is labeled '4/5' above it. The bar for 'vH2.16' reaches the 20 mark on the y-axis and is labeled '1/5' above it.

Immunization	Percent of Mice with Tumor
Vaccinia Vector	80 (4/5)
vH2.16	20 (1/5)

FIGURE 15C

A. Influenza Specific Cytolytic Activity of CD4+  
CD45RA+ Human T Cells Stimulated in the Presence  
of IL-12 and IL-18.

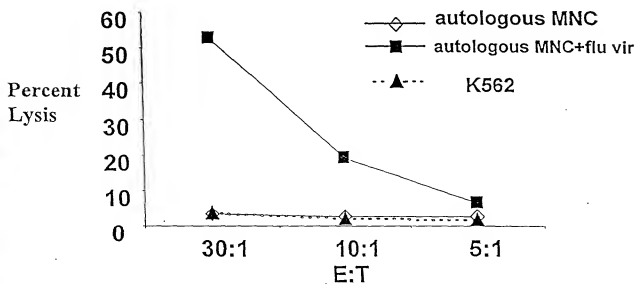
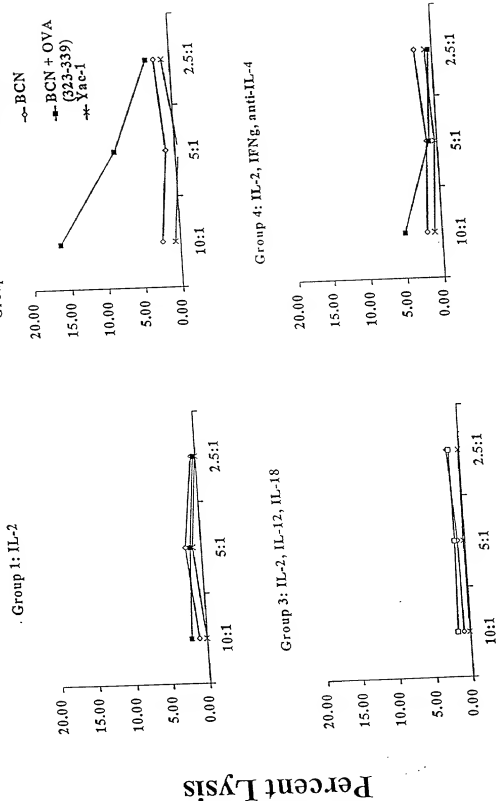


FIGURE 16

# CD4+ Primary Cytotoxic T Cell response



E:T

FIGURE 17

f1 ORIGIN

PHAGEMID CONTAINING  
GENE OF INTEREST  
WITHIN DOUBLE STRANDED  
cDNA LIBRARY

INFECT WITH  
M13KO7

SINGLE STRANDED  
cDNA LIBRARY

PCR WITH GENE  
SPECIFIC PRIMERS,  
MODIFIED WITH A  
BIOTIN MOLECULE  
OR A 5' PHOSPHATE

BIOTIN  
 $PO_4$   
DIGEST  
PHOSPHORYLATED  
STRAND WITH  
LAMBDA  
EXONUCLEASE TO  
GENERATE SINGLE  
STRANDED PROBE  
BIOTIN

HYBRIDIZE

BIOTIN

BIOTIN

STREPTAVIDIN-COATED  
PARAMAGNETIC BEADS

WASH

DENATURE, 0.1 N NaOH

REPAIR:

PRIME WITH SEQUENCE-SPECIFIC PRIMER  
AND EXTEND WITH TAQ DNA POLYMERASE

TRANSFORM  
DOUBLE STRANDED  
PHAGEMIDS INTO E.  
COLI

SCREEN COLONIES FOR  
GENE OF INTEREST

FIGURE 18

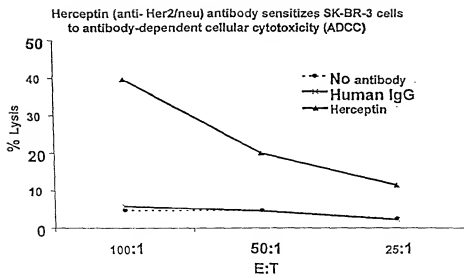


FIGURE 19

# Tolerance to Alloantigens Induced in presence of Antigens and Anti-CD40 Ligand Antibody

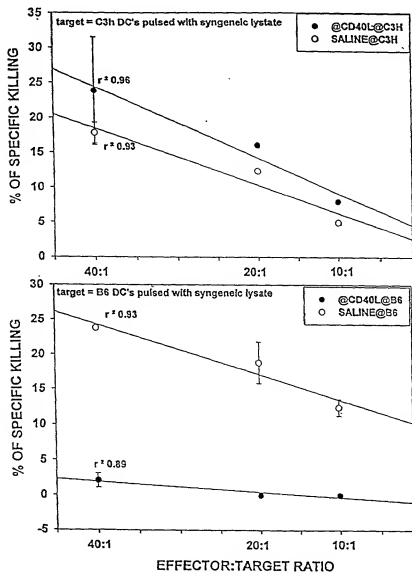


FIGURE 20

